Abrogation of antitumor effects of corynebacterium parvum and BCG by antimacrophage agents: brief communication

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Abrogation of Antitumor Effects of *Corynebacterium parvum* and BCG by Antimacrophage Agents: *Brief Communication* [1,2]

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ABSTRACT—The consistently demonstrable antitumor effect of *Corynebacterium parvum* and BCG against a 7,12-dimethylbenz[*a*]anthracene-induced rat fibrosarcoma, growing either as a localized subcutaneous tumor or in ascites form, was abrogated by treatment of rats with antimacrophage agents such as silica or carrageenan.—J Natl Cancer Inst 59: 1751-1753, 1977.

Numerous infectious agents, such as BCG (*1, 2*), *Corynebacterium* (*3*), *Toxoplasma* (*4*), or nematode parasites (*5*), as well as irritants such as endotoxin (*6*), peptone (*7*), glucan, or synthetic pyrans (*8, 9*), which can stimulate the mononuclear phagocyte system and potentiate the immune response against various antigens, also have considerable antitumor activity in vivo. Depending on the route and timing of administration of such agents, macrophages acquire the capacity to kill neoplastic cells in vitro with considerable selectivity (*8, 10-13*). Other observations imply an inverse relationship between the macrophage content of tumors and tumor regression (*14*) or metastasis (*15*). However, direct evidence that macrophages are involved in tumor resistance continues to be elusive.

It has recently been shown that treatment of the host with agents that diminish the functional capacities of macrophages in vitro markedly enhanced tumor growth in vivo (*16*). The present work demonstrates that the widely recognized antitumor activity of *Corynebacterium parvum* and BCG is entirely annulled by treatment with these same antimacrophage agents.

MATERIALS AND METHODS

Animals.—Inbred female DA rats, weighing 150–200 g, were used and maintained under conventional conditions.

Tumors.—Tumors were induced with DMBA and passaged in vivo as described in (*7*). One of these tumors, DMBA 12, which had progressively lost its immunogenicity during in vivo passages, acquired the capacity to grow, at least for a restricted number of passages, in ascites form. In these experiments, ascites tumor cells were injected either sc or ip into the DA rats.

BCG.—Living organisms of the Pasteur strain of BCG were obtained from the mycobacterial collection of the Trudeau Institute, Saranac Lake, New York (TMC #1011), stored in glass vials, and kept at −85° C until used in order to inhibit tumor growth (table 1).

C. parvum.—*C. parvum* organisms (strain 2683, Institut Pasteur) were cultured as described in (*17*). Heat-killed organisms were harvested by means of centrifugation, washed three times in 0.15 ml saline, and freeze dried. On the day indicated for each experiment, 3 mg of *C. parvum* was injected either sc or ip.

Antimacrophage agents.—Silica particles (Dörentrup quartz, #12; average diameter, 5 μ) were suspended in PBS and injected as indicated for each experiment. Carrageenan (Sea Kem 21; Marine Colloids, Inc., Springfield, N.J.) was dissolved in PBS by being heated to 100° C for 15 minutes in a water bath; rats were given 5 mg in PBS either iv or ip as indicated for each experiment.

RESULTS

The effects of *C. parvum* and/or BCG and of silica particles and/or carrageenan on the growth of local subcutaneous tumors and of ascites tumors were assessed separately.

Effects of *C. parvum* and/or BCG and of Silica Particles and/or Carrageenan on a Progressively Growing, Local Subcutaneous Tumor

The sc inoculation of 10^3–10^4 DMBA 12 tumor cells led to rapidly growing, local subcutaneous tumors; in the controls, tumors reached a considerable weight within 3 weeks (table 1). Pretreatment of rats with *C. parvum* or BCG distinctly diminished tumor growth. On the contrary, treatment on the day of tumor cell inoculation with carrageenan and particularly with silica particles significantly enhanced local tumor growth. In animals pretreated with *C. parvum* or BCG, the antitumor effect was fully reversed by silica or carrageenan

Abbreviations used: DMBA = 7,12-dimethylbenz[*a*]anthracene; PBS = phosphate-buffered saline.

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TABLE 1.—*Inhibition of the growth of subcutaneous tumors by BCG and/or* C. parvum *and its reversal by silica and/or carrageenan*

Tumor treatment[a]	Tumor weight, g[b]
Controls	16.8±2.9
10^8 BCG sc on day −5	7.4±3.7[c]
3 mg *C. parvum* sc on day −7	7.6±2.2[c]
10^8 BCG sc on day −5; 5 mg carrageenan iv on day 0	24.4±4.7[c]
10^8 BCG sc on day −5; 10 mg silica iv on day 0	21.8±4.4
3 mg *C. parvum* sc on day −7; 10 mg silica iv on day 0	26.3±8.3[d]
3 mg *C. parvum* sc on day −7; 5 mg carrageenan iv on day 0	21.8±3.5[d]
10 mg silica iv on day 0	26.8±4.9[c]
5 mg carrageenan iv on day 0	24.6±4.9[c]

[a] On day 0, 10^4 tumor cells were injected sc; the rats were killed and tumor weights were assessed on day 16.

[b] Each value represents the mean of 12 rats ±SD.

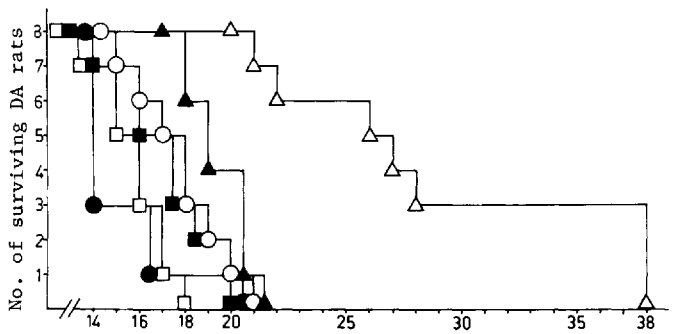
[c] Values are significantly different ($P<0.001$) from those for the controls, as determined by Student's *t*-test.

[d] Values are significantly different ($P<0.005$) from those for the controls, as determined by Student's *t*-test.

administered on day 0. Results such as those in table 1 have been obtained in several other experiments.

Effects of *C. parvum* and/or BCG and of Silica Particles and/or Carrageenan on Growth of Ascites Tumor

Results of a typical experiment are represented in text-figure 1. In controls, ip inoculation of 10^3 DMBA-induced syngeneic ascites tumor cells led to progressive tumor growth; within approximately 3 weeks, all rats died of tumors. In rats pretreated ip with 3 mg *C. parvum* on day −10, the survival time was prolonged by 1 to 3 weeks. This antitumor effect of *C. parvum* was consistently abrogated by silica and/or carrageenan administered on the day of tumor cell inoculation. Pretreatment with 10^8 BCG cells injected ip on day −7 led to similar increase in antitumor resistance, which was likewise abrogated by ip treatment of rats with the antimacrophage agents (not shown).



TEXT-FIGURE 1.—Inhibition of ascites tumor growth by pretreatment with *C. parvum* and abrogation of this effect by silica and/or carrageenan. ○, controls; △, 3 mg *C. parvum* was injected ip on day −10; □, 10 mg silica was injected ip on day 0; ●, 5 mg carrageenan was injected ip on day 0; ■, *C. parvum*+silica; ▲, *C. parvum*+carrageenan.

DISCUSSION

Antimacrophage agents, such as silica and carrageenan, have been shown to promote the growth of a syngeneic tumor of low immunogenicity (*16*); this finding indicates that in tumor-bearing rats these agents diminished innate resistance to tumors. The present work shows that in accordance with numerous earlier observations, microbial agents with adjuvant activity, such as *C. parvum* and BCG, consistently exert antitumor activity, especially when given prior to tumor implantation. The antitumor effects of these agents were similarly manifested in tumors growing subcutaneously and in the ascites form. Administration of silica or carrageenan on the same day as the tumor cell inoculum was injected cancelled out the beneficial effects of *C. parvum* or BCG. Antitumor resistance of rats pretreated with adjuvants and then given silica or carrageenan was frequently even lower than that in untreated controls, i.e., was similar to that in rats given silica or carrageenan alone. Since the present work was completed, similar observations have been reported by Hopper et al. (*18*).

In recent years, varied indirect evidence suggested a crucial role for macrophages in surveillance against neoplastic transformation [reviewed and discussed in (*19–21*)]. Diverse observations indicate that antitumor activity of various biologic and synthetic immunopotentiators, such as BCG, *C. parvum*, glucans, and pyrans, is mediated predominantly by mononuclear phagocytes. In in vitro situations, silica and carrageenan damage macrophages selectively; however, there are no analogous data to show that the in vitro effects of these agents also apply in vivo. After treatment with silica and/or carrageenan, a brief period of clearly diminished macrophage function can be discerned (*16*). Conceivably, this period, short as it is, could contribute to tumor implantation and initiation of progressive tumor growth. However, it is equally possible that tumor growth is enhanced by agents released from damaged macrophages (*16*). The present findings provide further evidence for the concept of an effective role for mononuclear phagocytes in natural tumor resistance, but in view of the complexity of the in vivo situation, they cannot be taken as a final proof for such a function of macrophages.

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